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DIAGNOSIS OF SPONGIFORM DISEASE

This invention relates to the detection of spongiform encephalopathy and other demyelinating conditions in mammals and is particularly, but not exclusively, concerned with the diagnosis of bovine spongiform encephalopathy (BSE).

BSE is a recent neurological disorder of cattle, which was first reported in the U.K. after 1982, following a change in the preparation of "bone and meal" feeds. BSE has attracted some public concern, lest it be transmitted to humans following meat consumption. It has been suggested that BSE is caused by "prions", a type of infectious protein.

The present invention is based on an alternative model of the genesis of various forms of spongiform encephalopathy and other demyelinating conditions in mammals. According to the proposed model, BSE and related diseases are conceived as autoimmune diseases arising as a result of molecular mimicry between certain infective agents and the myelin of the infected mammal. This new model of BSE, in particular, is based on the following experimental observations.

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A characteristic histopathological feature of BSE is a "spongiform" appearance, which also occurs in chronic but not acute "experimental allergic encephalomyelitis" (EAE), at least in rabbits and guinea pigs. A short sequence of bovine myelin (FSWGAEGQK), which withstands denaturation following heating to 100°C for one hour, was reported over twenty-five years ago to produce hind quarters paralysis, tremors and death, following inoculation into guinea pigs, which to some extent resembles the features observed in cattle suffering from BSE. In accordance with the present invention, this sequence has been used as a computer probe to search for proteins showing molecular mimicry. This sequence, in denatured form, may be described as encephalitogenic.

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Analysis of proteins in databases (Genbank and SwissProt) revealed that 3 microbes showed molecular mimicry of the bovine myelin sequence, the best one being found in 4-carboxy-muconolactone-decarboxylase of Acinetobacter calcoaceticus, a common microbe present in soil and water supplies. These sequence similarities are shown in the following Table.

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Comparison of amino acids of bovine myelin to microorganisms from Genbank and SwissProt which have similar sequences in other proteins.

Source	Amino acids	Positions	Locations
Bovine myelin	LSRFSWGAE	110 - 118	
Acinetobacter calcoaceticus	ISRFAWGEV	41 - 49	4-carboxy-muconolactone decarboxylase
Agrobacter tumefaciens	YTRFTWGAP	693 - 701	Beta-glucosidase
Ruminococcus albus	YTQFEISAE	274 - 282	Beta-glucosidase

Alphabetic letters refer to biochemical symbols for amino acids.

In conformity with the new model, it has now been found that sera of BSE affected cattle contain significantly high levels of antibodies to Acinetobacter species.

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The present invention therefore provides a diagnostic test for spongiform encephalopathy and other demyelinating conditions in mammals which comprises assaying antibodies present in the mammal which bind to an antigenic peptide which exhibits molecular mimicry of a mammalian myelin peptide, especially one having the sequence FSWGAEQK. The term "molecular mimicry" refers to a degree of similarity (sequence homology) as between the antigenic peptide and a myelin peptide which results in the formation of antibodies which cross-react with myelin and demyelinate nervous tissue. The presence of such antibodies at elevated levels compared to

Preparation of bacteria

Enzyme-linked immunosorbent assay

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ELISA METHOD SHEET

1. Dilute antigen in coating buffer, add 200 μ l to each well. Incubate overnight at 4°C wrapped in foil.
2. Wash out the antigen, using washing/incubation buffer; the wells of the tray should be completely full during the washing stages as the Tween-20 prevents any further protein from being absorbed onto the plastic. Wash 3 times, leaving for approx. 4 minute intervals at room temperature.
3. Incubate the plate at 37°C for 1hr with 0.2% Ovalbumin in washing/incubation buffer.
4. Add 200 μ l of test serum. Dilutions are made in washing/incubation buffer. Incubate for 2 hours at 37°C wrapped in foil.
5. Repeat washing process as in 2.
6. Add 200 μ l Horseradish peroxide HRP-conjugated second antibody, also diluted in washing/incubation buffer.
7. Repeat washing process as in 2.
8. Add 200 μ l substrate (ABTS) to wells; leave to develop colour for approx. 20 minutes in the dark at room temperature. Stop reaction with 100 μ l of stopping solution and read plate at 630nm.

RESULTS

Antibodies to A. calcoaceticus of total immunoglobulin (IgG + IgA + IgM) were significantly elevated in the BSE sera (mean \pm SE: 0.99 ± 0.05) when compared to CVL controls (0.65 ± 0.06) ($t = 4.48$, $p < 0.001$), organic farming controls aged more than 30 months (0.57 ± 0.03) ($t = 7.19$, $p < 0.001$) and organic farming controls aged less than 30 months (0.53 ± 0.02) ($t = 8.64$, $p < 0.001$). These results are shown in the attached Figure.

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Legend to figure:

Antibody titres (bar = mean) for 30 controls aged less than 30 months ($A < 30m$), 28 controls aged more than 30 months ($A > 30m$), 18 controls from the Central Veterinary Laboratory (CVL) compared to 29 BSE sera, when tested against Acinetobacter calcoaceticus (Figure 1a) and E. coli (Figure 1b). (Dashed line represents 95% confidence limits for mean of controls: $A < 30m + A > 30m$ - one tailed test) (OD = optical density).

There was no significant difference between the CVL controls and the organic farming controls aged more than 30 months, but there was a small, statistically significant difference with the sera from animals aged less than 30 months ($t = 2.41$, $p < 0.05$). A re-examination of the CSL control serum with the highest anti-Acinetobacter level of 1.16 OD, showed that it came from a clinically normal control animal, diagnosed as negative to BSE on the statutory diagnostic criteria, and it was also negative when tested for scrapie associated fibrils. This case did however have white matter vacuolation of the substantia nigra and internal capsule, although this had been seen before and not considered significant.

One clear result from these studies, is that in at least in one "transmissible spongiform encephalopathy" (TSE), namely BSE, a specific immune response can be demonstrated against a microbe that is found readily in the environment of cattle and which also happens to possess a molecular sequence resembling bovine myelin.

Other forms of spongiform encephalopathy including Creutzfeldt Jacob disease (CJD) and Multiple Sclerosis (MS) are open to explanation on the same model as indicated for BSE. CJD sera and MS sera are currently under test to confirm the presence of cross-reacting antibodies.

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